RESEARCH ARTICLE

Pre-operative Predictive Factors for Intra-operative Pathological Lymph Node Metastasis in Rectal Cancers

Chun Gao*, Jing-Tao Li, Long Fang, Si-Wei Wen, Lei Zhang, Hong-Chuan Zhao*

Abstract

Background: A number of clinicopathologic factors have been found to be associated with pathological lymph node metastasis (pLNM) in rectal cancer; however, most of them can only be identified by expensive high resolution imaging or obtained after surgical treatment. Just like the Child-Turcotte-Pugh (CTP) and the model for end-stage liver disease (MELD) scores which have been widely used in clinical practice, our study was designed to assess the pre-operative factors which could be obtained easily to predict intra-operative pLNM in rectal cancer. Methods: A cohort of 469 patients who were treated at our hospital in the period from January 2003 to June 2011, and with a pathologically hospital discharge diagnosis of rectal cancer, were included. Clinical, laboratory and pathologic parameters were analyzed. A multivariate unconditional logistic regression model, areas under the curve (AUC), the Kaplan-Meier method (log-rank test) and the Cox regression model were used. Results: Of the 469 patients, 231 were diagnosed with pLNM (49.3%). Four variables were associated with pLNM by multivariate logistic analysis, age<60 yr (OR=1.819; 95% CI, 1.231-2.687; *P*=0.003), presence of abdominal pain or discomfort (OR=1.637; 95% CI, 1.052-2.547; *P*=0.029), absence of allergic history (OR=1.879; 95% CI, 1.041-3.392; *P*=0.036), and direct bilirubin≥2.60 μmol/L (OR=1.540; 95% CI, 1.054-2.250; *P*=0.026). The combination of all 4 variables had the highest sensitivity (98.7%) for diagnostic performance. In addition, age<60 yr and direct bilirubin≥2.60 μmol/L were found to be associated with prognosis. Conclusion: Age, abdominal pain or discomfort, allergic history and direct bilirubin were associated with pLNM, which may be helpful for preoperative selection.

Keywords: Rectal cancer - lymph node metastasis - predictive factors - allergic history - direct bilirubin

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Introduction

Lymph node metastasis (LNM) is an important indicator of oncologic outcome for patients with rectal cancer (Chen et al., 2012; Peng et al., 2013). Studies have demonstrated that the number of retrieved lymph nodes is significantly associated with relapse and survival rates (Tepper et al., 2001; Kim et al., 2009). The inability to examine a sufficient number of lymph nodes may lead to failure in identifying metastatic lymph nodes, and thus portend a worse prognosis (Tsai et al., 2011). However, lymph node dissection might impair genitourinary functions and it is not appropriate to be performed for those patients without LNM risk (Ishida et al., 2012). Therefore, preoperative selection of patients at high risk of LNM is very important in the surgical treatment.

Now, preoperative imaging has been used to provide some important information for the most risk factors: T-stage, N-stage, and circumferential resection margin (CRM) (Gosens et al., 2007; Lambregts et al., 2011). Endoluminal ultrasound (EUS) is traditionally used for T-staging, whereas magnetic resonance imaging (MRI) has recently been used for assessment of the primary tumor and CRM (Group, 2006). However, unfortunately, EUS, computed tomography, and MRI all lack sufficient accuracy for identifying nodal metastases (Bipat et al., 2004; Lahaye et al., 2005), and the detection of metastatic nodes remains difficult. Despite advances in high resolution imaging, under-staging of the nodal status occurs in up to 16% on MRI, and 25% on EUS (Lambregts et al., 2011). In addition, some of these techniques are not available for most patients in developing countries, such as China.

Previously published literatures had assessed the clinicopathologic factors associated with LNM and found some risk factors, including age<60 years, tumor diameter, tumor location, depth of invasion, poor differentiation, lymphovascular invasion and perineural invasion (Bayar et al., 2002; Ricciardi et al., 2006; Wu et al., 2007; Fujita et al., 2009; Kim et al., 2011; Ding et al., 2011; Chang et al., 2012; Saraste et al., 2013). But most of these factors could be obtained after surgical treatment and should not regarded as predicting factors. They are also not helpful for preoperative selection of patients at high risk. Considering

*Department of Gastroenterology, China-Japan Friendship Hospital, Ministry of Health, Beijing, China *For correspondence: hongchuanzhao@hotmail.com, gaochun@bjmu.edu.cn*

that Child-Turcotte-Pugh (CTP) and the model for end-stage liver disease (MELD) score, which consists of a few simple variables, including serum bilirubin, albumin, creatinine and International Normalized Ratio, and have been used as a reliable measurement for preoperative risk stratification and mortality risk assessment in cirrhotic patients undergoing surgery (Hoteit et al., 2008), we hope that we could find some similar predicting factors in rectal cancer.

Our study was designed to assess the pre-operative factors which could be obtained easily before surgical treatment to predict intra-operative pathological lymph node metastasis (pLNM) in rectal cancer, which may potentially be helpful for preoperative selection of patients with high risk of pLNM.

Materials and Methods

Study population

A cohort of 469 patients who were treated at our hospital in the period from January 2003 to June 2011, and with a pathologically hospital discharge diagnosis of rectal adencarcinoma, were included in our study, including 231 patients (49.3%) with intra- operative pLNM and 238 patients without pLNM. Patients who followed these criteria would be excluded: 1) those who had been treated by any method at inclusion or with pathological diagnosis for more than 15 days; 2) those who did not have a whole pathological data, including palliative operation and those whose operation were performed at other hospitals; 3) those who died after surgery for less than three months; 4) those who had a presence of other malignancies, including leukemia and lymphoma; 5) those who had been treated by immunosuppressive agents and cytotoxic drugs in the past six months; 6) those who were diagnosed with familial adenomatous polyposis, inflammatory bowel disease and hereditary non-polyposis colorectal cancer; and 7) those who had a presence of serious disease of other important organs or systems, and rheumatic diseases. The study was approved by the Human Research Ethics Committee of our hospital and it was in accordance with the principles of the Declaration of Helsinki.

Clinical, laboratory and pathological parameters

Pre-operative basic characteristics were used to determine the predicting factors of pLNM, including demographic data (gender, age and body mass index/ BMI), chief compliant (abdominal pain or discomfort, hematochezia, change in bowel habit, and change in stool character), past history and personal history (hypertension, allergic history, drinking and smoking), vital signs (body temperature, heart rate, respiratory rate and blood pressure), and laboratory examination (white blood cell, hemoglobin, platelet count, total bilirubin, direct bilirubin, ALT, albumin, creatinine, serum sodium, potassium, carcinoembryonic antigen and CA19-9). The pathological data included macroscopic growth type, tumor differentiation, pathological tumor classification (pT), pathological lymph node stage (pN), pathological distant metastasis (pM), status of lymph node removal (lymphadenectomy), maximum tumor diameter, and presence or absence of necrosis and vascular invasion.

Follow-Up

Patients were followed after surgery by serial clinical examination and CEA assessment every 3 months during the first year, every 6 months during the second year, and annually thereafter. Thoracoabdominal computed tomography (CT) scanning was performed every 6 months for the first 2 years. Colonoscopy was performed after 1 year and 3 to 5 years thereafter, depending on individual patient risk. If recurrence was suspected, then further diagnostic methods were used as required.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS version 16.0; Chicago, Ill, USA) was used for data management and statistical analyses. Categorical variables were compared using the chi-square test and the Fisher exact test. Continuous variables were compared using an Independent-Samples Student's t-test; and the nonparametric Mann-Whitney U test, as appropriate, was used to determine significance. According to results of univariate analysis, multivariate unconditional logistic regression model was used to determine the independently pre-operative predicting factors for pLNM. For clinical practice, continuous variables would be changed as categorical variables, and the cutoff values were determined based on their receiver operator characteristics (ROC) curves, areas under the curve (AUC), sensitivity, specificity and Youden index. After that, logistic regression analysis was repeated. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Youden index, Kappa coefficients and AUC were used to determine the diagnostic performance of predicting factors. We used the Kaplan-Meier method (log-rank test) and multivariate Cox regression model to determine the prognostic significance of pLNM, possible predicting factors alone and combination. Stepwise multiple regression analysis (Backward: Wald; Entry: 0.05, Removal: 0.10) was used. We expressed results as odds ratios (ORs) and their 95% confidence intervals (CIs). For all tests, $P<0.05$ was considered statistically significant and all P values quoted are two-sided.

Results

Study population and basic characteristics

Four hundred and sixty-nine patients pathologically diagnosed with rectal cancer were included in our study, including 231 patients with intra-operative pathological lymph node metastasis (pLNM) (49.3%) and 238 patients without pLNM. Their basic and pathological characteristics were shown in Table 1-2. The mean age was 62.1±12.6 years and 286 patients were male (61.0%). Hematochezia was the most common chief complaint (88.5%). Fifty-eight patients (12.4%) had allergic history and 129 (27.5%) were smoking. The mean number of lymph node removal (lymphadenectomy) was 13.3± 8.4 (median, 12). In the 231 patients with pLNM, 136 were diagnosed with lymph node stage 1 (pN1) and 95 patients were diagnosed with pN2 (41.1%).

Table 1. Pre-operative Basic Characteristics of 469 Patients with Rectal Adenocarcinoma (RAC)

Characteristic	Total patients (n=469)*	RAC patients with LNM (n=231)*	RAC patients without LNM (n=238)*	*P* Value
Demographic				
Male (n, %)	286 (61.0)	146 (63.2)	140 (58.8)	0.331
Age (yr)	62.1±12.6	60.6±12.8	63.6±12.2	**0.012**
BMI[a] (kg/m^2)	23.95±3.54	23.90±3.67	24.0±3.41	0.779
Chief compliant				
Abdominal pain or discomfort (n, %)	115 (24.5)	67 (29.0)	48 (20.2)	**0.026**
Hematochezia (n, %)	415 (88.5)	204 (88.3)	211 (88.7)	0.907
Change in bowel habit (n, %)	281 (59.9)	149 (64.5)	132 (55.5)	**0.046**
Change in stool character (n, %)	318 (67.8)	161 (69.7)	157 (66.0)	0.387
Past history and personal history				
Allergic history (n, %)	58 (12.4)	20 (8.7)	38 (16.0)	**0.016**
Hypertension (n, %)	131 (27.9)	58 (25.1)	73 (30.7)	0.179
Alcohol intake (n, %)	29 (6.2)	18 (7.8)	11 (4.6)	0.154
Smoking (n, %)	129 (27.5)	55 (23.8)	74 (31.1)	0.077
Vital signs				
Body temperature (°C)	36.52±0.28	36.55±0.29	36.50±0.27	**0.043**
Heart rate (bpm)	76±8	77±8	76±8	0.489
Respiratory rate (breaths/min)	19±1	19±1	19±1	0.164
Mean artery pressure (mmHg)	95±11	95±11	96±11	0.406
Laboratory examination				
White blood cell (×9/L)	6.66±2.10	6.70±2.09	6.61±2.11	0.666
Hemoglobin (g/L)	132±20	131±20	132±19	0.553
Platelet count (×9/L)	233±79	235±74	232±84	0.659
Total bilirubin[†] (μmol/L)	10.26 (7.70-14.40)	10.26 (7.40-15.0)	10.26 (8.13-13.68)	0.451
Direct bilirubin[†] (μmol/L)	2.30 (1.60-3.42)	2.50 (1.70-3.42)	2.10 (1.40-3.42)	**0.025**
ALT[†] (U/L)	16 (12-22)	15 (12-22)	16 (12-22)	0.262
Albumin level[b] (g/L)	42.3±4.5	42.3±4.4	42.2±4.6	0.908
Creatinine (mg/dL)	82.1±18.0	80.8±15.5	83.3±20.1	0.128
Serum sodium[c] (mmol/L)	141±3.0	141±3.1	142±2.9	**0.028**
Serum potassium[c] (mmol/L)	4.1±0.5	4.1±0.4	4.1±0.5	0.282
Carcinoembryonic antigen[d†] (ng/ml)	4.33 (2.22-11.91)	5.40 (2.40-13.95)	3.50 (2.08-8.67)	**0.009**
Serum CA19-9[e†] (kU/L)	14.02 (7.20-28.63)	15.10 (7.39-33.91)	13.41 (7.10-23.10)	0.109

RAC, rectal adenocarcinoma; LNM, lymph node metastasis; BMI, body mass index; Data were available in [a]403 (202+201), [b]463 (227+236), [c]457 (226+231), [d]392 (192+200) and [e]370 (178+192) patients. The numbers before the brackets indicate the total available cases in the two groups. *Plus-minus value indicates mean±standard deviation; [†]Median (inter-quartile range, Q1 – Q3)

Univariate analysis: comparison of rectal cancer patients with and without intra-operative pathological lymph node metastasis

Chi-square test, Student's t-test and Mann-Whitney U test were used to compare the differences between patients with and without pLNM. As shown in Table 1, compared with patients without pLNM, the patients with pLNM had a younger age (*P*=0.012), higher percentages of presence of abdominal pain or discomfort (*P*=0.026) and change in bowel habit (*P*=0.046), a lower percentage of allergic history (*P*=0.016), an increased body temperature (*P*=0.043), increased levels of direct bilirubin (*P*=0.025) and CEA (*P*=0.009), and a lower value of serum sodium (*P*=0.028).

For the intra-operative pathological characteristics (Table 2), lymph node metastasis was associated with macroscopic growth type (*P*=0.004), tumor differentiation (*P*<0.001), pathological tumor classification (pT) (*P*<0.001), and presence of vascular invasion (*P*<0.001). No statistically differences were shown by univariate analysis for distant metastasis (pM) (*P*=0.454) and the maximum tumor diameter (*P*=0.079).

Multivariate analysis: pre-operative predicting factors for pathological lymph node metastasis in rectal cancer

Multivariate unconditional logistic regression model was used to determine the independently pre-operative predicting factors for pLNM. When 8 variables were included based on the results of univariate analysis, data were available for 385 patients and 3 variables were shown as statistically differences. After CEA was excluded considering that the statistical difference was not found in Supplemental Table 1 by multivariate analysis, 457 patients were included and logistic regression analysis was repeated. Statistically differences were shown for 5 variables, including abdominal pain or discomfort (OR=1.620; 95% CI, 1.037-2.533; *P*=0.034), allergic history (OR=0.493; 95% CI, 0.269-0.902; *P*=0.022), body temperature (OR= 2.131; 95% CI, 1.063-4.274; *P*=0.033), direct bilirubin (OR=1.146; 95% CI, 1.028-1.279; *P*=0.014), and serum sodium (OR=0.932; 95% CI, 0.873-0.995; *P*=0.034).

For clinical practice, continuous variables were changed as categorical variables, and the cutoff values were determined based on the combination of sensitivity, specificity Youden index, ROC curves and AUC (data not shown). Four variables remained as statistically different by logistic regression (Table 3), indicating as predicting factors, including age<60 yr (OR=1.819; 95% CI, 1.231-2.687; *P*=0.003), presence of abdominal pain or discomfort (OR=1.637; 95% CI, 1.052-2.547; *P*=0.029), absence of allergic history (OR=1.879; 95% CI,

Table 2. Intra-operative Pathological Characteristics of 469 RAC Patients

Characteristic	Total patients (n=469)*	RAC patients with LNM (n=231)*	RAC patients without LNM (n=238)*	*P* Value
Macroscopic growth type				
Ulcer type (n, %)	295 (62.9)	155 (67.1)	140 (58.8)	**0.004**
Non-ulcer type (n, %)	84 (17.9)	29 (12.6)	55 (23.1)	---
Unknown (n, %)	90 (19.2)	47 (20.3)	43 (18.1)	---
Tumor differentiation				
Moderately/well (n, %)	388 (82.7)	166 (71.9)	222 (93.3)	**<0.001**
Poorly (n, %)	57 (12.2)	46 (19.9)	11 (4.6)	---
Unknown (n, %)	24 (5.1)	19 (8.2)	5 (2.1)	---
Pathological tumor classification (pT)				**<0.001**
pT1 (n, %)	18 (3.8)	1 (0.4)	17 (7.1)	---
pT2 (n, %)	90 (19.2)	21 (9.1)	69 (29.0)	---
pT3 (n, %)	118 (25.2)	54 (23.4)	64 (26.9)	---
pT4 (n, %)	243 (51.8)	155 (67.1)	88 (37.0)	---
Pathological lymph node stage (pN)				**<0.001**
pN0 (n, %)	238 (50.7)	0 (0.0)	238 (100.0)	---
pN1 (n, %)	136 (29.0)	136 (58.9)	0 (0.0)	---
pN2 (n, %)	95 (20.3)	95 (41.1)	0 (0.0)	---
Pathological distant metastasis (pM)				
pM0 (n, %)	427 (91.0)	208 (90.0)	219 (92.0)	0.454
pM1 (n, %)	42 (9.0)	23 (10.0)	19 (8.0)	---
Number of lymph node				
Lymph node-positive (metastasis) (Median)	2.2±4.5 (0)	4.5±5.5 (3)	---	---
Total number of lymph node removal (lymphadenectomy) (Median)	13.3±8.4 (12)	15.0±9.1 (13)	11.6±7.3 (11)	**<0.001**
Mean of maximum tumor diameter[a] (cm)	4.4±1.7	4.6±1.8	4.3±1.6	0.079
Presence of necrosis (n, %)	13 (2.8)	8 (3.5)	5 (2.1)	0.369
Presence of vascular invasion (n, %)	55 (11.7)	47 (20.3)	8 (3.4)	**<0.001**
Results of follow-up				
Death (n, %)	72 (15.4)	46 (19.9)	26 (10.9)	**0.007**
Survival (n, %)	277 (59.1)	118 (51.1)	159 (66.8)	**0.001**
Lost (n, %)	120 (25.6)	67 (29.0)	53 (22.3)	0.095
Time of follow-up[†] (months)	24.0(9.0-44.3)	20.0(9.0-37.9)	29.1(10.5-48.5)	**0.013**

Data were available in [a]426 (208+218) patients. The numbers before the brackets indicate the total available cases in the two groups. *Plus-minus value indicates mean±standard deviation; [†]Median (inter-quartile range, Q1 – Q3)

1.041-3.392; *P*=0.036), and direct bilirubin≥2.60 μmol/L (OR=1.540; 95% CI, 1.054-2.250; *P*=0.026).

Diagnostic performance for prediction of pathological lymph node metastasis using pre-operative basic characteristics

We used the sensitivity, specificity, PPV, NPV, Kappa coefficients, Youden index and the AUC to demonstrate the diagnostic performance for these parameters, including age<60 yr (Age) alone, absence of allergic history (AAH) alone, presence of abdominal pain or discomfort (APD) alone, direct bilirubin≥2.60 μmol/L (TBil) alone, and the combination of both, three and four variables for prediction of intra-operative pLNM. For each variable, the sensitivity was 91.3% for AAH, 45.9% for Age, 29.0% for APD, and 49.4% for TBil, whereas the specificity was 16.0%, 68.5%, 79.8%, 59.7%, respectively. After combination, the highest sensitivity was 98.7% for the combination of AAH, Age, APD and TBil, whereas the highest specificity was 54.6% for the combination of Age and APD.

As the sensitivity increased, the specificity decreased. Taking into account sensitivity and specificity, combination of Age and APD yielded the highest Youden index and corresponding AUC (the sensitivity was 62.8% whereas the specificity was 54.6%). To make a long story short, for the diagnostic performance, combination of the four variables had the highest sensitivity (98.7%), APD alone had the highest specificity (79.8%), and combination of Age and APD had the highest AUC (0.587).

Table 3. Multivariate Analysis for Pre-operative Predicting Factors of Pathological Lymph Node Metastasis in Rectal Adenocarcinoma*

Variable/predicting factor	Adjusted OR	95% CI	*P* Value
Age<60 yr	1.819	1.231-2.687	**0.003**
Abdominal pain or discomfort	1.637	1.052-2.547	**0.029**
Change in bowel habit	----	----	0.136
Absence of allergic history	1.879	1.041-3.392	**0.036**
Body temperature≥36.7 °C	----	----	0.119
Direct bilirubin≥2.60 μmol/L	1.540	1.054-2.250	**0.026**
Serum sodium≤141 mmol/L	----	----	0.125

CI, confidence intervals; OR, odds ratios; *457 patients were included because the data were not available for 12 patients. The four continuous variables (age, body temperature, direct bilirubin and serum sodium) were changed as categorical variables

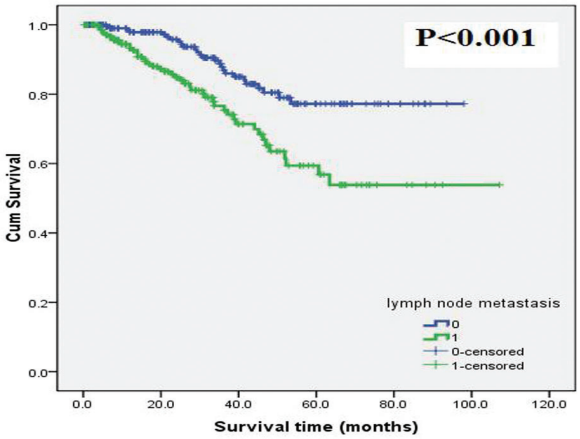

Figure 1. Kaplan-Meier Curve (log rank test) Showed That Lymph Node Metastasis was Associated with the Prognosis (survival time)

CEA and CA19-9 had been regarded as two important biomarkers in rectal cancer. We compared the diagnostic performance of CEA and CA19-9 with our variables. As shown in Table 1 by univariate analysis, patients with pLNM had a higher level of CEA (*P*=0.009) and no difference was found for CA19-9 (*P*=0.109). When CEA was included in multivariate analysis, no statistical difference was found (OR=1.001; 95% CI, 0.999-1.003; *P*=0.251). The value of 5ng/ml was determined as cutoff value for CEA, based on the range of normal value and previously published literatures (Shin et al., 2012). The sensitivity, specificity and AUC of CEA were 43.7%, 67.6% and 0.557, respectively, indicating that our variables were superior to CEA and CA19-9.

Follow-up and survival analysis

The median follow-up was 24.0 months (range, 0.4-107.1 months; inter-quartile range, 9.0-44.3 months). One hundred and twenty patients (25.6%) were lost, including 67 in the group with pLNM and 53 in those without pLNM (*P*=0.095). Of the total, 72 (15.4%) patients were dead, statistical analysis (Table 2) showed that the percentage of the death was higher in patients with pLNM than in patients without pLNM (*P*=0.007). Kaplan-Meier

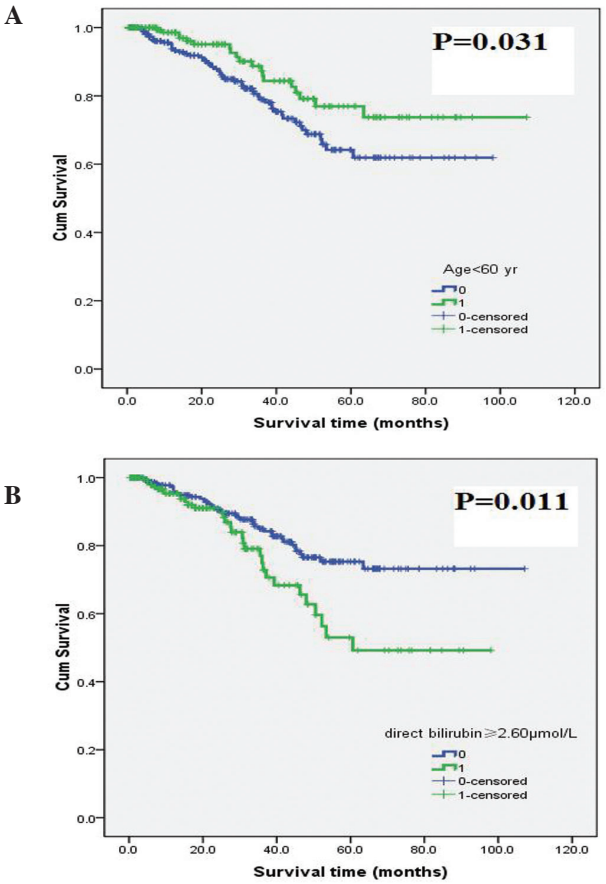

Figure 2. Age<60 yr (A) and Direct Bilirubin≥2.60 μmol/L (B) Were Shown to be Associated with the Prognosis by the Kaplan-Meier Curves (log rank test)

method and the log-rank test were used to determine the prognostic significance of pLNM (Figure 1), the four above-mentioned pre-operative predicting factors alone and combination. For the four variables alone (Figure 2), age<60 yr (P=0.031) and direct bilirubin≥2.60 μmol/L (P=0.011) were significantly associated with prognosis (survival time). For the combination of both, three and four variables, no significant differences were found, including combination of Age and APD (P=0.127) and combination of AAH, Age, APD and TBil (P=0.892). The results remained unchanged when the Cox regression analysis was performed.

Discussion

Our study showed that age<60 yr, presence of abdominal pain or discomfort, absence of allergic history, and direct bilirubin≥2.60 μmol/L were associated with pLNM in rectal cancer. They could be obtained easily before surgical treatment and expected to be helpful for prediction of intra-operative pLNM. Combination of these four factors had the highest sensitivity of 98.7%, indicating that they could potentially be used for preoperative selection of patients with high risk, whereas presence of abdominal pain or discomfort alone had the highest specificity (79.8%). In addition, survival analysis showed that age<60 yr and direct bilirubin≥2.60 μmol/L were related to the prognosis.

This study was designed to determine the simply pre-operative predicting factors for intra-operative pLNM in rectal cancer, although some previously published literatures had assessed the clinicopathologic factors associated with LNM (Bayar et al., 2002; Ricciardi et al., 2006; Wu et al., 2007; Fujita et al., 2009; Kim et al., 2011; Ding et al., 2011; Chang et al., 2012; Saraste et al., 2013). These studies found some factors associated with LNM, including age<60 years, tumor diameter, tumor location, depth of invasion, poor differentiation, lymphovascular invasion and perineural invasion. As shown in Table 2, most of them were also found in our study, including macroscopic growth type, tumor differentiation, depth of invasion/pathological tumor classification (pT), and presence of vascular invasion. However, they were obtained after the surgical treatment and could only be regarded as associated or risk factors, not predicting factors. And they could also not be helpful for preoperative selection of patients with high risk of pLNM in rectal cancer.

In the 4 predicting factors, for the first time, presence of abdominal pain or discomfort, absence of allergic history, and direct bilirubin≥2.60 μmol/L were demonstrated to be associated with intra-operative pLNM in rectal cancer, whereas age<60 yr had been shown by two studies (Ding et al., 2011; Kim et al., 2011). One study (Kim et al., 2011) reported that age<60 yr (P=0.02) was an independent risk factor of proximal lymph node involvement (PLNp) in patients with node positive rectal cancer. Patients with PLNp had poorer oncologic outcomes than those without PLNp in terms of overall survival (P<0.001). This conclusion was supported by another study which was performed (Ding et al., 2011) to identify risk factors of LNM in T2 rectal cancer, and showed that age was an independent predictor for overall LNM. Previous studies on other carcinomas (Singh et al., 2004; Caywood et al., 2005), such as breast cancer, also reported lower LNM rates in older patients.

Higher than normal levels of direct or indirect bilirubin may indicate different types of liver problems. Total bilirubin, one of the simple indexes of liver function, has been included in the CTP and MELD score which had been used as a reliable measurement for preoperative risk stratification and mortality risk assessment in cirrhotic patients undergoing surgery (Hoteit et al., 2008). Moreover, direct bilirubin was recently included as one of the five variables in a prediction rule which was developed to stratify risk for pancreatic cancer in chronic pancreatitis patients with focal pancreatic mass lesions with prior negative endoscopic ultrasound guided fine needle aspiration (EUS-FNA) cytology (Cai et al., 2011). However, until now no information was available about the association of total, direct or indirect bilirubin with LNM, which was shown in our study for the first time.

Twenty-nine patients (6.2%) in our study were diagnosed with liver metastasis and no patients were diagnosed with pancreas involvement. In the 29 patients, 19 were in the group with pLNM and 10 were in the group without pLNM (19/231=8.2% vs. 10/238 =4.2%, P=0.071), indicating that the role of direct bilirubin in pLNM of rectal cancer can not be deduced from liver metastasis and pancreas involvement. In addition, some

other variables of liver function (total bilirubin, ALT and albumin) and two important tumor biomarkers (CEA and CA19-9) were included in our study, but no significant differences were shown. Moreover, direct bilirubin≥2.60 μmol/L was found to be associated with poor prognosis, which strengthened the role in pLNM of rectal cancer. The underlying mechanism remains as yet unclearly and further studies are required.

Allergic history was for the first time associated with pLNM in rectal cancer and the reasons has not yet clarified, whereas the association of abdominal pain or discomfort with pLNM could be understood based on the basic principals of occurrence and development of carcinoma. With the development of cell and molecular biology, molecular biomarkers have been expected to play important roles in predicting intra-operative pLNM in rectal cancer, such as chromosomal copy number alterations, epithelial-mesenchymal transition biomarkers and support vector machine guided model (Chen et al., 2012; Fan et al., 2012). High resolution imaging, for example gadofosveset-enhanced MRI has been showing high reproducibility and significantly improved accuracy for nodal staging and restaging in rectal cancer (Lambregts et al., 2011). However, they could not be used widely in clinical practice and are not available for most patients in developing countries.

Some limitations should also be acknowledged. The first limitation was the limited generalizability, because the entire study population came from one single tertiary referral hospital and extending these results to the overall population may be a concern. We would validate the results and conclusion in more rectal cancer patients in our hospital, and we expect that they could also be validated in greater numbers of patients and more referral hospitals. The second was that the median follow-up was 24.0 months (range, 0.4-107.1 months; inter-quartile range, 9.0-44.3 months) which was relatively shorter, and the slightly higher percentage of the lost patients (25.6%). They would be improved in our further studies. The third was that some potential risk factors, for example diabetes mellitus which has been put forward recently (Yuhara et al., 2011; Gao et al., 2013; Zhang et al., 2013), had not been included in our study. The reason was that these factors had not been considered in the beginning of design and implementation of our study.

In conclusion, our studies showed that four pre-operative predicting factors, including age<60 yr, presence of abdominal pain or discomfort, absence of allergic history and direct bilirubin≥2.60 μmol/L, were associated with intra-operative pathological lymph node metastasis in rectal cancer; and they could potentially be helpful for preoperative selection of patients with high risk of pLNM.

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